CLAIMS

What is claimed is:

1. A method of identifying a gene product having activity in a biosynthetic pathway, the method comprising:

a) producing a test cell by introducing into a genetically modified host cell an exogenous nucleic acid comprising a nucleotide sequence encoding a candidate gene product, wherein the genetically modified host cell produces a biosynthetic pathway intermediate, which intermediate is produced in an amount effective to inhibit growth of the genetically modified host cell; and

b) determining the effect, if any, of expression of the candidate gene product on growth of the test cell, wherein a reduction in growth inhibition indicates the candidate gene product has activity in a biosynthetic pathway involving the biosynthetic pathway intermediate.

2. The method of claim 1, wherein the exogenous nucleic acid comprises nucleotide sequences encoding two or more gene products.

3. The method of claim 1, wherein the exogenous nucleic acid is isolated from a cell of a species that is different from the genetically modified host cell.

4. The method of claim 3, wherein the exogenous nucleic acid is isolated from a eukaryotic cell or a prokaryotic cell.

5. The method of claim 3, wherein the exogenous nucleic acid is isolated from a cell of an organism selected from a protozoan, a plant, a fungus, an alge, a yeast, a reptile, an amphibian, a mammal, a marine microorganism, a marine invertebrate, an arthropod, an isopod, an insect, an arachnid, an archaebacterium, and a eubacterium.

6. The method of claim 3, wherein the genome of the organism is mutated prior to isolation of nucleic acid from the organism.

7. The method of claim 1, wherein the exogenous nucleic acid is a cDNA.

8. The method of claim 1, wherein the exogenous nucleic acid is a cDNA library.

9. The method of claim 1, wherein the exogenous nucleic acid is genomic DNA.

10. The method of claim 1, wherein the exogenous nucleic acid is a genomic DNA library.

11. The method of claim 1, wherein the exogenous nucleic acid is synthetic DNA.

12. The method of claim 11, wherein the synthetic DNA is amplified using a polymerase chain reaction.

13. The method of claim 1, wherein the genetically modified host cell is genetically modified with a nucleic acid comprising a nucleotide sequence encoding a biosynthetic pathway enzyme, wherein synthesis of the enzyme in the genetically modified host cell results in conversion of a substrate for the enzyme into the biosynthetic pathway intermediate.

14. The method of claim 1, wherein the genetically modified host cell is a prokaryotic cell.

15. The method of claim 14, wherein the prokaryotic cell is *Escherichia coli*.

16. The method of claim 1, wherein the genetically modified host cell is a eukaryotic cell.

17. The method of claim 16, wherein the eukaryotic cell is a yeast cell.

18. The method of claim 17, wherein the yeast cell is *Saccharomyces cerevisiae*.

19. The method of claim 1, further comprising isolating the exogenous nucleic acid from the test cell.

20. The method of claim 1, further comprising separating growth-inhibited test cells from test cells that exhibit a reduction in growth inhibition by buoyant density separation; and isolating the exogenous nucleic acid from the test cells that exhibit reduced growth inhibition.

21. The method of claim 1, wherein the biosynthetic pathway is a terpene biosynthetic pathway, and wherein the intermediate is a prenyl diphosphate.

22. The method of claim 21, wherein the genetically modified host cell converts acetyl-CoA into a prenyl diphosphate.

23. The method of claim 22, wherein the genetically modified host cell is genetically modified with one or more nucleic acids that comprise nucleotide sequences encoding one or more of acetoacetyl-CoA thiolase, hydroxymethylglutaryl-CoA synthase, hydroxymethylglutaryl-CoA reductase, mevalonate kinase, phosphomevalonate kinase, and mevalonate pyrophosphate decarboxylase.

24. The method of claim 22, wherein the genetically modified host cell is genetically modified with one or more nucleic acids that comprise nucleotide sequences encoding one or more of acetoacetyl-CoA thiolase, hydroxymethylglutaryl-CoA synthase, hydroxymethylglutaryl-CoA reductase, mevalonate kinase, phosphomevalonate kinase, mevalonate pyrophosphate decarboxylase, and isopentenyl pyrophosphate isomerase.

25. The method of claim 22, wherein the genetically modified host cell is genetically modified with one or more nucleic acids that comprise nucleotide sequences encoding acetoacetyl-CoA thiolase, hydroxymethylglutaryl-CoA synthase, hydroxymethylglutaryl-CoA reductase, mevalonate kinase, phosphomevalonate kinase, mevalonate pyrophosphate decarboxylase, isopentenyl pyrophosphate isomerase, and a prenyl-transferase.

26. The method of claim 21, wherein the genetically modified host cell converts mevalonate to a prenyl diphosphate.

27. The method of claim 26, wherein the genetically modified host cell is genetically modified with one or more nucleic acids comprising nucleotide sequence encoding mevalonate kinase, phosphomevalonate kinase, and mevalonate pyrophosphate decarboxylase, and wherein the genetically modified host cell is cultured in the presence of mevalonate.

28. The method of claim 26, wherein the genetically modified host cell is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding mevalonate kinase, phosphomevalonate kinase, mevalonate pyrophosphate decarboxylase, and isopentenyl pyrophosphate isomerase, and wherein the genetically modified host cell is cultured in the presence of mevalonate.

29. The method of claim 26, wherein the genetically modified host cell is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding mevalonate kinase, phosphomevalonate kinase, mevalonate pyrophosphate decarboxylase, isopentenyl pyrophosphate isomerase, and a prenyl transferase, and wherein the genetically modified host cell is cultured in the presence of mevalonate.

30. The method of claim 21, wherein the prenyl diphosphate is a monoprenyl diphosphate.

31. The method of claim 21, wherein the prenyl diphosphate is a polyprenyl diphosphate.

32. The method of claim 31, wherein the polyprenyl diphosphate is selected from geranyl diphosphate, farnesyl diphosphate, geranylgeranyl diphosphate, geranylfarnesyl diphosphate, hexaprenyl diphosphate, heptaprenyl diphosphate, octaprenyl diphosphate, solanesyl diphosphate, and decaprenyl diphosphate.

33. The method of claim 21, wherein the genetically modified host cell converts 1-deoxy-D-xylulose-5-phosphate to a prenyl diphosphate.

34. The method of claim 33, wherein the prenyl diphosphate is a monoprenyl diphosphate.

35. The method of claim 33, wherein the prenyl diphosphate is a polyprenyl diphosphate.

36. The method of claim 33, wherein the genetically modified host cell is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding 1-deoxy-D-xylulose-5-phosphate synthase, 1-deoxy-D-xylulose-5-phosphate reductoisomerase, 4-diphosphocytidyl-2-C-methyl-D-erythritol synthase, 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase, 2*C*-methyl-D-erythritol 2,4-cyclodiphosphate synthase, 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate synthase, and isopentenyl/dimethylallyl diphosphate synthase.

37. The method of claim 33, wherein the genetically modified host cell is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding 4-diphosphocytidyl-2-C-methyl-D-erythritol synthase, 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase, 2*C*-methyl-D-erythritol 2,4-cyclodiphosphate synthase, 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate synthase, and isopentenyl/dimethylallyl diphosphate synthase.

38. The method of claim 37, wherein the test cell is grown in the presence of methylerithritol.

39. The method of claim 1, wherein the determining step is by monitoring optical density of a liquid culture comprising the test cell, or by identifying a viable test cell.

40. A method of identifying a gene product having activity in a biosynthetic pathway, the method comprising:

a) producing a plurality of test cells by introducing into a plurality of genetically modified host cells a plurality of exogenous nucleic acids, each comprising a nucleotide sequence encoding a candidate gene product, wherein the genetically modified host cells produce a biosynthetic pathway intermediate, which intermediate is produced in an amount effective to inhibit growth of the genetically modified host cell; and

b) determining the effect, if any, of expression of the candidate gene products on growth of the test cells, wherein a reduction in growth inhibition identifies a candidate gene

product having activity in a biosynthetic pathway involving the biosynthetic pathway intermediate.

41. A genetically modified host cell, wherein the host cell is genetically modified with a nucleic acid comprising a nucleotide sequence encoding a biosynthetic pathway enzyme, wherein synthesis of the enzyme in the host cell results in conversion of a substrate for the enzyme into a biosynthetic pathway intermediate, which intermediate is produced in an amount effective to inhibit growth of the genetically modified host cell.

42. The genetically modified host cell of claim 41, wherein the host cell is a prokaryotic cell.

43. The genetically modified host cell of claim 41, wherein the host cell is a eukaryotic cell.

44. The genetically modified host cell of claim 43, wherein the host cell is a plant cell.

45. The genetically modified host cell of claim 41, wherein the genetically modified host cell converts acetyl-CoA into a prenyl diphosphate.

46. The genetically modified host cell of claim 45, wherein the genetically modified host cell is genetically modified with one or more nucleic acids that comprise nucleotide sequences encoding acetoacetyl-CoA thiolase, hydroxymethylglutaryl-CoA synthase, hydroxymethylglutaryl-CoA reductase, mevalonate kinase, phosphomevalonate kinase, and mevalonate pyrophosphate decarboxylase.

47. The genetically modified host cell of claim 46, wherein one or more of the nucleotide sequences is operably linked to an inducible promoter.

48. The genetically modified host cell of claim 46, wherein one or more of the nucleotide sequences is operably linked to a constitutive promoter.

49. The genetically modified host cell of claim 41, wherein the genetically modified host cell converts mevalonate into a prenyl diphosphate.

50. The genetically modified host cell of claim 45, wherein the genetically modified host cell is genetically modified with one or more nucleic acids that comprise nucleotide sequences encoding mevalonate kinase, phosphomevalonate kinase, and mevalonate pyrophosphate decarboxylase.

51. The genetically modified host cell of claim 50, wherein one or more of the nucleotide sequences is operably linked to an inducible promoter.

52. The genetically modified host cell of claim 50, wherein one or more of the nucleotide sequences is operably linked to a constitutive promoter.

53. The genetically modified host cell of claim 41, wherein the host cell converts 1-deoxy-D-xylulose-5-phosphate to a prenyl diphosphate.

54. The genetically modified host cell of claim 53, wherein said host cell is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding 1-deoxy-D-xylulose-5-phosphate synthase, 1-deoxy-D-xylulose-5-phosphate reductoisomerase, 4-diphosphocytidyl-2-C-methyl-D-erythritol synthase, 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase, 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase, 1-hydroxy-2-methyl-2-(E)-butenyl 3-diphosphate synthase, and isopentenyl diphosphate/dimethylallyl diphosphate isomerase.

55. The genetically modified host cell of claim 41, wherein the nucleic acid is maintained extrachromosomally.

56. The genetically modified host cell of claim 41, wherein the nucleic acid is integrated into the genome of the host cell.

57. A library of genetically modified host cells, wherein the host cells are genetically modified with one or more nucleic acids that comprise nucleotide sequences encoding one or more terpene biosynthetic pathway enzymes and a prenyl transferase,

wherein the library comprises a plurality of member genetically modified host cells, and wherein each member is genetically modified with a nucleic acid comprising a nucleotide sequence encoding a different prenyl transferase.

58. A kit comprising a genetically modified host cell of claim 41.

59. The kit of claim 58, wherein at least one nucleotide sequence encoding a biosynthetic pathway enzyme is operably linked to an inducible promoter, and wherein the kit further comprises an inducer that activates the promoter.

60. A kit comprising the genetically modified host cell library of claim 57.

61. The kit of claim 60, further comprising a positive control cell that synthesizes a terpene synthase.

62. A method of identifying an agent that inhibits a metabolic pathway in a cell, the method comprising:

a) contacting a test cell with a test agent, wherein the test cell produces a metabolic pathway intermediate, which intermediate is produced in an amount effective to inhibit growth of the test cell; and

b) determining the effect, if any, of the agent on growth of the test cell, wherein a reduction in growth inhibition indicates the agent inhibits the metabolic pathway.

63. The method of claim 62, wherein the agent is an exogenous nucleic acid that is introduced into the test cell, wherein the exogenous nucleic acid comprises a nucleotide sequence encoding a candidate gene product, and wherein said determining step comprises determining the effect, if any, of expression of the candidate gene product on growth of the test cell, wherein a reduction in growth inhibition indicates the candidate gene product inhibits a metabolic pathway involving the metabolic pathway intermediate.